Pathogens Present in Acute Mangled Extremities From Afghanistan and Subsequent Pathogen Recovery

Timothy E. Wallum; Lt Col Heather C. Yun, USAF MC†; Maj Elizabeth A. Rini, USAF MC‡; LT Kristina Carter, MSC USN§; Charles H. Guymon*; MAJ Kevin S. Akers, MC USA*; MAJ Stuart D. Tyner, MS USA*; LTC Christopher E. White, MC USA*; COL Clinton K. Murray, MC USA†*

ABSTRACT Given the changing epidemiology of infecting pathogens in combat casualties, we evaluated bacteria and fungi in acute traumatic wounds from Afghanistan. From January 2013 to February 2014, 14 mangled lower extremities from 10 explosive-device injured casualties were swabbed for culture at Role 3 facilities. Bacteria were recovered from all patients on the date of injury. Pathogens recovered during routine patient care were recorded. The median injury severity score was 29, median initial Role 3/4 blood product support was 32 units, and median evacuation time was 42 minutes to first surgical care. Gram-positive bacteria were found in some wounds but not methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant *Enterococcus*. Most wounds were colonized with low-virulence, environmental gram-negative bacteria, and not recovered again during therapy, reflecting wound contamination. Only one wound had the same bacteria (*E. cloacae*) throughout care at the Role 3, 4, and 5 facilities. Three cultures from two patients had multidrug-resistant bacteria (*E. cloacae*, *E. coli*), all detected at Role 5 facilities. Molds were not detected at Role 3, whereas one patient had a mold at Role 4 and 5. Mangled lower extremity injuries have a high contamination rate with environmental organisms, which are not typically associated with infections during the course of the patient's care.

INTRODUCTION

Improved care of combat-related injured personnel through improved body armor, point of injury care, rapid evacuation, and far-forward surgical care has improved the mortality rate; however, there is substantial morbidity for those who survive.[1–3] Infections associated with combat-related injuries occur in approximately 25% of those arriving to the three largest U.S. military treatment facilities with the rate being as high as 50% if the patients are admitted to the intensive care unit (ICU).[3] The most commonly reported infections are in the skin and soft tissues (18%) with extremity injuries being the most common injury in Iraq and Afghanistan.[4–7] This is despite aggressive combat-related extremity injury infection prevention guidelines focusing on antimicrobial therapy and surgical wound management.[8]

In the civilian literature, severity of injured extremity open fractures correlates with increasing rates of infection with tibia infection rates as high as 39% with a 10% amputation rate.[9–15] Typical infecting bacteria of open fractures include *Staphylococci* and gram-negative rods.[9,16–19] Within the U.S. military, infection rates of open fractures of the tibia have been reported to be 27% with an amputation rate of 22%.[20] Most studies took place in Iraq, showing high frequencies of gram-negative rods including *Acinetobacter baumannii*.[20,21]

Studies performed to determine the origin of these bacteria show that the majority result from nosocomial transmission; however, these data were primarily for *Acinetobacter*, with limited data from Afghanistan or for other pathogens.[22] Since that time, with the increasing numbers of casualties in Afghanistan, the predominant colonizing and infecting pathogen has transitioned to extended-spectrum β-lactamase–producing (ESBL) *Enterobacteriaceae* with increasing rates of invasive mold infections.[23,24]

To characterize the new epidemiology of pathogens from Afghanistan, attempts have been made in recent studies to evaluate whether patients are colonized with ESBL bacteria before injury, are directly inoculated at the time of injury, or are acquired via nosocomial transmission, as likely occurs with *Acinetobacter*. Initial screening for multidrug resistance (MDR) revealed an 11% colonization rate with ESBL-producing *Escherichia coli* among healthy deployed personnel in Afghanistan.[25] Despite the introduction of MDR pathogens into the facility, there does not appear to be substantial clonality between infecting or colonizing *E. coli* isolates.[26,27] However, to date, no surveillance study has been performed in Afghanistan to replicate the acute wound colonization studies that were carried out during the Vietnam War or during the early years of the war in Iraq.[28,29]

This study assessed wound colonization among patients with mangled lower extremities at the time of initial

*United States Army Institute of Surgical Research, 3698 Chambers Pass, JBSA Fort Sam Houston, TX 78234.

†San Antonio Military Medical Center, 3551 Roger Brooke Drive, JBSA Fort Sam Houston, TX 78234.

‡Landstuhl Regional Medical Center, CMR 402, Box 1559, APO AE 09180, Landstuhl, Germany.

§Naval Environmental Preventive Medicine Unit 5 San Diego, 3235 Albacore Alley, San Diego, CA 92136.

doi: 10.7205/MILMED-D-14-00301

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 JAN 2015	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Pathogens Present in Acute Mangled Extremities From Afghanistan and Subsequent Pathogen Recovery	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Wallum T. E., Yun H. C., Rini E. A., Carter K., Guymon C. H., Akers K. S., Tyner S. D., White C. E., Murray C. K.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Institute of Surgical Research, JBSA Fort Sam Houston, Tx 78234	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**SAR**	**7**	

presentation to NATO Role 3 facilities with cultures pre- and postamputation and then serially through the patient's medical care until discharge from a Role 5 facility. It includes evaluation of surgical and antimicrobial therapy. Given the high rate of infections and wound contamination of distal lower extremity mangled injuries, these high-risk patients were selected to best answer this question regarding the new epidemiology of pathogens from Afghanistan in infections of the skin, bone, and soft tissue.

METHODS

Patient Population

Between January 2013 and February 2014, patients who arrived at the Role 3 facilities in southwest Afghanistan, Kandahar Airfield (KAF) or Bastion, with a traumatically mangled lower extremity as a result of blast or ballistic injury, were evaluated for enrollment. Those who were unlikely to undergo amputation, non-U.S. service members or patients managed at another facility before arrival at the Role 3 facility were excluded. Patients underwent verbal consent if they were able to effectively communicate. Otherwise, informed consent was waived, in accordance with Institutional Review Board (U.S. Army Medical Research and Material Command Office of Research Protection) approval, given the minimal risk of the study.

Clinical Data

Data collected at the time of presentation included each patient's general demographics, injury characterization, and initial clinical parameters. In addition, time from injury to Role 3 presentation, time to surgery, duration of surgery, antimicrobial management, and blood product support were collected. Follow-on clinical information and outcomes were collected from various sources including the Department of Defense Trauma Registry, the Patient Administration and Biostatistics Activity, the Composite Heath Care System, the military clinical inpatient documentation (Essentris), and the Armed Forces Health Longitudinal Technology Application.

Specimen Collection and Microbiological Assessment

Lower leg wound injury sites underwent swabbing (BactiSwab with Amies media (gel) without charcoal, from Remel) pre- and postamputation at the first Role 3 facility to which the patient arrived (Bastion or KAF), and were stored at 4°C for shipment to the microbiology lab at the U.S. Army Institute of Surgical Research (USAISR). The specimens arrived at the USAISR a median of 15 days (min–max, 5–41) from time of injury and all cultures were set up within 48 hours of receipt of specimen. Swabs were streaked onto 5% Sheep's blood and MacConkey agar culture plates and then placed in thioglycollate broth on arrival (i.e., within 12 hours) to the microbiology laboratory. All isolates were then identified using routine clinical microbiology methods. Any molds, yeast, or suspected anaerobes collected at initial swabbing were referred to the clinical microbiology section at San Antonio Military Medical Center. Bacterial or fungal specimens collected as part of routine patient care at Bagram Airfield (BAF), Landstuhl Regional Medical Center (LRMC) Germany, or U.S military treatment facilities underwent identification per routine clinical microbiological laboratory standard operating procedures at those facilities and were not available for further microbiological analysis in this study. MDR was defined as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and ESBL *Enterobacteriaceae*.

RESULTS

Between January 2013 and February 2014, seven Soldiers and three Marines were enrolled in the study (Table I). They were all males with a median age 23 years (min–max, 19–30), and all were injured by dismounted improvised explosive devices with three suffering minor burns of less than 2% full-thickness. All survived to discharge from military treatment facilities in the United States.

Prehospital care during evacuation to the first Role 3 facility included six patients receiving blood products (1–4 units), three receiving crystalloids, and four receiving tranexamic acid (patients 1, 7, 9, 10). The median time to Role 3 was 42 minutes (min–max, 31–290) with two patients (subjects 6 and 7) arriving more than 250 minutes after injury. The median time to first infusion of antimicrobial after arrival was 9 minutes (min–max, 5–75) and the median time from antibiotics to first surgery was 42 minutes (min–max, 25–49). Only one patient had documented prehospital antibiotics (patient 1). The median time from arrival at Role 3 to first surgery was 50 minutes (min–max, 35–75) and median time from injury to first surgery including evacuation time was 102 minutes (min–max, 77–325).

Patients 1 and 10 arrived hypothermic (93.4°F and 89.6°F, respectively) while the median temperature of all patients was 97.4°F (min–max, 89.6–99.0°F). The median systolic blood pressure was 80 mm Hg (min–max, 60–94) with median hemoglobin on arrival in the emergency department of 12.2 g/dL (min–max, 7.8–14.9), median platelet count of 222 ($\times 10^3/\mu L$) (min–max, 107–483), median pH of 7.26 (min–max, 7.04–7.49), median base deficit of −10 (mEq/L) (min–max −3 to −21), and median international normalization ratio of 1.4 (min–max, 1.2–2.3)

Blood product support through Role 4 military treatment facility, duration of the first surgery at Role 3, median number of ICU days and median hospital days through Role 4, median number of times that wounds underwent incision and drainage through Role 4, and average time between wound incision and drainage procedures are shown in Table II.

Antimicrobial therapy on arrival or during initial Role 3 surgery was primarily cefazolin at KAF, or amoxicillin/clavulanate at Bastion, typically with continuation of the same antimicrobial therapy during care at the first Role 3 (Table III). Antimicrobial therapy was expanded to include a

TABLE I. Wound Patterns, Injury Severity, and Amputation Status of Patients From Southwestern Afghanistan Who Underwent Lower Extremity Amputation

Patient	ISS Role 3	First Role 3 Facility	Initial Level of LEA[a]	Revised Level of LEA[b]	Upper Extremity Amputated (L/R)	Wrist Amputation	Digit Amputation (*n*)	Other Upper Extremity Wounds[c]	Perineum, Pelvic Injury	Penetrating Abdominal Injury
1	14	KAF	R-BKA	BKA				Y	Y	N
2	14	KAF	L-BKA	TKA	L	Y		Y	Y	N
3	33	KAF	L-TKA	AKA	R	N	2	Y	N	N
			R-TKA	AKA						
4	59	KAF	L-TKA	TKA				N	Y	N
			R-BKA	TKA						
5	24	KAF	R-TKA	TKA				Y	Y	N
6	41	KAF	R-TKA	TKA	L/R	N	1/1	Y	Y	Y
7	45	KAF	L-AKA	AKA				Y	Y	N
			R-BKA	TKA						
8	24	Bastion	R-BKA	BKA				Y	Y	N
9	14	Bastion	R-BKA	BKA	L	N	2	Y	N	N
10	36	Bastion	L-BKA	TKA				Y	Y	Y
			R-AKA	AKA						

ISS, injury severity score; LEA, lower extremity amputation; AKA, above knee amputation; TKA, through knee amputation; BKA, below knee amputation. [a]Indicates the initial level of the amputation, after first surgery at Role 3 facility. [b]Indicates the revised level of the amputation, as of discharge from Role 4 facility. At discharge, patient number 6 had a TKA but expected to have an AKA stateside. Patient 10 had AKA revised to a right hip disarticulation. [c]Indicates whether or not the patient had wounds on the upper extremities, in addition to the amputation. All patients had lower extremity wounds, in addition to the lower extremity amputations.

TABLE II. Blood Product Support, Durations of Surgery, ICU Days, and Ventilated Days Through Discharge From the Role 4 Medical Treatment Facility

Patient	Total Blood Products (Units)	PRBC (Units)	FFP (Units)	Platelets (Units)	Whole Blood (Units)	Cryoprecipitate (Units)	Crystalloid (mL)	EBL (mL)	First Surgery at Role 3 (Min)	Second Surgery at Role 3 (Min)	ICU (Days)	Ventilated (Days)
1	22	11	10	1	0	0	8,525	1,950	170	100	7	1
2	30	12	16	2	0	0	6,200	820	64	58	5	1
3	23	13	9	1	0	0	15,700	700	94	51	7	1
4	107	36	34	7	0	30	12,845	300	174	301	8	8
5	89	33	30	6	0	20	9,250	2,400	253	71	3	3
6	33	17	13	3	0	0	9,962	1,300	265	298	6	3
7	34	17	14	3	0	0	1,1065	1,250	222	205	7	3
8	8	5	3	0	0	0	8,885	150	272	197	6	0
9	29	14	10	4	0	1	8,469	200	277	124	5	0
10	120	52	46	10	7	5	4,972	1,300	418	128	6	5
Median	31.5	15.5	13.5	3	0	0	9067.5	1,035	237.5	126	6	2

PRBC, packed red blood cells; FFP, fresh frozen precipitate; EBL, estimated blood loss at time of surgery; ICU, intensive care unit.

carbapenem in 1 patient for suspected sepsis at the second Role 3 facility in Afghanistan (Bagram). On reaching the Role 4 facility in Germany, antimicrobial coverage was expanded for two patients to include antifungal therapy. Therapy was narrowed in some patients and broadened in others. Antimicrobial beads were implanted in wounds at Bagram for subjects 1 through 4, typically using 5 to 10 antibiotic beads containing a 1:1 ratio (g:g) of tobramycin and cefazolin. Topical Dakin's solution of 0.0025% was instilled via negative-pressure wound therapy device, typically 50 or 60 cc every 1 to 2 hours on the ward in subjects 1, 5, 6, 7, and 9 while at BAF.

Although most wounds appeared to clinically improve after care at the first and second Role 3 facilities in Afghanistan, some patients had necrotic tissue noted at the Role 4 facility. This included the left and right lower extremity of subject 3 on postinjury days 4 to 6. Subject 4 had fungus noted on the postinjury day 5 debridement. Subject 5 had necrosis on postinjury day 2, and subject 6 continued to have pus and debris in his wounds on postinjury day 4.

Of the 14 wounds that underwent amputation at the Role 3 facility, 13 were swabbed with 11 having both pre- and post-amputation cultures and two with only preamputation cultures (Table IV). At Role 3, two wounds had matching

TABLE III. Antimicrobials Given by Time Postinjury and Military Treatment Facility Through Role 4

Patient	On Arrival or During First Surgery	First Role 3 (Bastion or KAF)	Second Role 3 (BAF)	Role 4 (LRMC)
1	Amoxicillin/Clavulanate, Cefazolin	PID 0: Cefazolin	PID 1: Cefazolin, Levofloxacin	PID 2 to PID 7: Cefazolin
2	Cefazolin	PID 0: Cefazolin, PID 1: Clindamycin	PID 2 to 3: Clindamycin	PID 4 to PID 6: Clindamycin
3	Cefazolin	PID 0 to 1: Cefazolin, Piperacillin/Tazobactam	PID 2: Meropenem, Metronidazole, PID 3: Cefazolin, Tobramycin, Vancomycin	PID 4 to PID 7: Cefazolin
4	Cefazolin, Piperacillin/Tazobactam	PID 0: Cefazolin	PID 1: Cefazolin, PID 1 and 2: Clindamycin	PID 3 to PID 8: Meropenem, Vancomycin, Amphotericin B, Voriconazole
5	Cefazolin	PID 0: Cefazolin	PID 1: Cefazolin, Vancomycin	PID 2 to PID 3: Cefazolin
6	Cefazolin, Metronidazole	PID 0: Piperacillin/Tazobactam	PID 1: Cefazolin, Levofloxacin, Metronidazole	PID 2 to 3: Cefazolin, Levofloxacin, PID 4 to 6: Cefazolin
7	Cefazolin	Cefazolin, Levofloxacin	PID 1: Cefazolin	PID 2 to 10: Cefazolin
8	Amoxicillin/Clavulanate	Amoxicillin/Clavulanate	PID 1: Amoxicillin/Clavulanate, PID 2: Cefazolin	PID 3 to 6: Cefazolin
9	Amoxicillin/Clavulanate	PID 0 to 1: Amoxicillin/Clavulanate	PID 2: Cefazolin	PID 3 to 5: Cefazolin
10	Amoxicillin/Clavulanate	PID 0: Amoxicillin/Clavulanate	PID 1: Piperacillin/Tazobactam, Cefazolin	PID 1 to 6: Vancomycin, Piperacillin/Tazobactam, Voriconazole, Amphotericin B

PID, postinjury day; KAF, Kandahar Airfield; BAF, Bagram Airfield; LRMC, Landstuhl Regional Medical Center.

TABLE IV. Bacterial and Fungal Isolates Recovered From 14 Lower Extremity Wounds That Underwent Amputation

Patient	Injury Site	Role	Organisms Isolated
1	RLE	3	*Enterococcus* spp (PID 0/pre-), *Burkholderia cepacia* (PID 0/post-)
2	LLE	3	*Pseudomonas oryzihabitans* (PID 0/pre-), *Sphingomonas paucimobilis* (PID 0/pre-), *Kloeckera* spp (PID 0/post-)
		4	*Aspergillus niger* (PID 5)
3	LLE	3	*P. stutzeri* (PID 0/pre-), *P. fluorescens* (PID 0/pre-), CNS (PID 0/pre-), *Streptococcus viridans* group (PID 0/post-)
		4	*Penicillium* spp (PID 5), *Aspergillus* spp (PID 5), *Enterococcus hirae* (PID 5), *Bacillus* spp (PID 5)
		5	*P. aeruginosa* (PID 8), *Escherichia coli* (PID 8) ESBL-producing
	RLE	3	*P. fluorescens* (PID 0/pre- and post-), *Streptococcus D non-enterococcus* (PID 0/pre-), *P. oryzihabitans* (PID 0/post-)
4	LLE	4	Mucorales order (PID 3), *A. flavus* (PID 3), *A. terreus* (PID 3), *Fusarium* spp (PID 3), *Bacillus* spp (PID 3), *Enterococcus faecium* (PID 5), *Stenotrophomonas maltophilia* (PID 6), *Corynebacterium* spp (PID 6)
		5	Nonseptate fungi (PID 10)
	RLE	3	*P. fluorescens* (PID 0/pre- and post-), *Aggregatibacter aphrophilus* (PID 0/pre-), *Aeromonas salmonicida* (PID 0/post-)
		4	Zygomycete (Mucoraceae) (PID 3), *E. faecium* (PID 5)
		5	*Saksenaea erythrospora* (PID 12), *Fusarium* spp (PID 59), *S. maltophilia* (PID 12)
5	RLE	3	Gram-Positive rods (GPR) (PID 0/pre- and post-), *P. fluorescens* (PID 0/pre-), *Enterococcus* spp (PID 0/pre-)
		5	*E. coli* (PID 58) ESBL-producing, *Enterobacter cloacae* (PID 58) ESBL-Producing, CNS (PID 61), *Staphylococcus aureus* (PID 89)
6	RLE	3	*Enterococcus* spp (PID 0/pre-), Resembles *Rhodotorula* spp (PID 0/pre-)
7	LLE	3	GPR (PID 0/pre- and post-)
	RLE	3	GPR (PID 0/pre-), *Micrococcus* spp (PID 0/pre-), Resembles *Rhodotorula* spp (PID 0/pre-), Yeast (PID 0/post-)
		4	*Alternaria* spp (PID 3), *Penicillium* spp (PID 3), Sterile Septated Hyaline Hyphomycete (PID 3)
8	RLE	3	*P. fluorescens* (PID 0/pre-), CNS (PID 0/post-), GPR (PID 0/post-)
9	RLE	3	GPR (PID 0/pre-), *P. fluorescens* (PID 0/pre-), *P. putida* (PID 0/pre-), *E. cloacae* (PID 0/pre-)
		4	*E. cloacae* (PID 4)
		5	*E. cloacae* (PID 17)
10	LLE	3	*Enterococcus* spp (PID 0/pre-), *P. fluorescens* (PID 0/pre-)
	RLE	3	*Enterococcus* spp (PID 0/pre-), *B. cepacia* (PID 0/pre-), *P. fluorescens* (PID 0/pre-)
		4	*Alternaria* spp (PID 1), *Penicillium* spp (PID 1), *Aspergillus* spp (PID 1), *Enterococcus faecium* (PID 1)
		5	*E. coli* (PID 7)

LLE, left lower extremity; RLE, right lower extremity; PID, postinjury day; pre-, culture of mangled extremity just before surgical amputation; post-, culture of amputation stump immediately after surgical amputation; CNS, coagulase-negative *Staphylococci*.

pre- and postamputation cultures (both yielding *Pseudomonas fluorescens*) and two wounds had pre- and postamputation gram-positive rods that were not further identified. No Role 3 isolate matched an organism recovered at a Role 4 or 5 culture, except for subject 9 who had an *Enterobacter cloacae* (not ESBL-producing) recovered at Role 4 and 5 and subject 10 who had *Enterococcus* recovered at Role 3 and 4 but not 5. There were no matching isolates between Role 4 and 5 except for subject 4 who had a mold recovered at Role 4 and 5 in both extremities (with genus/species not clearly matching) and subject 9 who had the *E. cloacae* at all 3 Roles.

Among the gram-positive pathogens recovered from the patient, there were no methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant *Enterococcus*. Although *Enterococcus* was recovered at Role 3 and 4 in 9 wounds, it was not recovered at Role 5. Only 1 gram-positive isolate, methicillin-susceptible *S. aureus*, was recovered at Role 5.

Among the gram-negative pathogens, the most commonly recovered pathogens were non–*Pseudomonas aeruginosa* (*P. fluorescens* [10], *P. stutzeri* [1]), *P. oryzihabitans* [2], *P. putida* [1]). These were all recovered pre-Role 5, whereas the only *P. aeruginosa* isolate was recovered at a Role 5 facility. Among intrinsically resistant organisms, *Burkholderia cepacia* was recovered twice at Role 3 and *Stenotrophomonas maltophilia* at Role 4 and 5. Other gram-negative bacteria recovered at Role 3 that are known to be associated with environmental exposure include *Sphingomonas paucimobilis*, *Aeromonas salmonicida*, and *Aggregatibacter aphrophilus*. Three cultures from two wounds had MDR pathogens, namely ESBL-producing *E. coli* and *E. cloacae*, all of which were recovered at Role 5 facilities along with the intrinsically resistant *S. maltophilia*.

Among fungal pathogens, yeasts (including *Kloeckera* spp and likely *Rhodotorula* spp) were recovered four times (from 3 wounds) at Role 3 facilities but not at Role 4 or 5 facilities. Molds were not recovered at Role 3 despite being queried by appropriate cultures in the swabs returned to the United States, but were found in six wounds (5 subjects) at Role 4 and 5 facilities. Four of those wounds had mold at Role 4 without subsequent recovery at Role 5 facilities. Two wounds (both in subject 4) had molds found at Role 4 and again at Role 5. Patients frequently had numerous types of molds in their wounds including *Aspergillus* spp, *Penicillium* spp, Mucorales order, and *Fusarium* spp.

There was no clear relationship with pathogen type or Role of recovery based upon injury severity score (ISS), laboratory parameters, blood product support, evacuation times, antimicrobial therapy, or surgical timing on inspection.

DISCUSSION

Increasing survival rates during the wars in Iraq and Afghanistan have resulted in increased morbidity of patients, including the development of severe skin and soft-tissue infections with MDR pathogens. This has required the military medical system to respond by seeking new strategies to diagnose, treat, and prevent the development of infections. A goal of improved care is to continually assess clinical management of injuries and to re-evaluate the changing epidemiology of wound infections and their causative pathogens. This study highlights the presence of environmental organisms contaminating wounds at the time of injury with the development of MDR bacteria later in the patient's clinical course. In addition, the presence of mold was not detected near the time of injury, but appeared in cultured wounds later in the clinical course at Role 4 and 5 facilities. Overall, this study also confirms that wound sampling should not be done early after injury as it is not predictive of future infecting pathogens. In addition, pathogens are regionally time frame–dependent necessitating ongoing microbiological epidemiological surveillance.

Our data show that initial wound-colonizing bacteria are low-virulence environmental contaminants that do not result in sustained infections under these treatment conditions and do not include MDR bacteria. Although there were fewer skin contaminants, such as coagulase-negative *Staphylococcus*, than in the previous study in Iraq and Afghanistan, there were more gram-negative bacteria in the wounds.[29,30] This might reflect the higher severity of injury in this study versus the previous study in Iraq. The predominant gram-negative bacteria at Role 5 are similar to the acute wound study from the Vietnam War that revealed a transition to increasing gram-negative bacteria from time of injury over 5 days.[28] MDR bacteria were detected in two of 14 wounds (14%) at Role 5, which is similar to other studies regarding the type and rate of MDR bacteria among extremity injuries.[20,21] The bacteria were not *Acinetobacter baumannii* as previously seen at Role 4 and 5 facilities during combat operations in Iraq, but instead were *Enterobacteriaceae* pathogens including *E. coli* and *E. cloacae*. Given that these may colonize even healthy persons without an injury, the source might be the patient themselves, through either previous colonization or selection with ongoing antimicrobial therapy.[23,25,31] The French military has noted a 35% ESBL-producing *E. coli* fecal colonization rate among soldiers evacuated from Afghanistan.[32] A study assessing soft-tissue infection among U.K. military personnel undergoing amputations revealed infections with *Aeromonas hydrophila* (12% or 17% of limb wounds) were associated with more proximal amputations and a greater number of debridements.[33] Interestingly, *A. hydrophila* is associated with leech therapy and two patients in our study received leech therapy at Role 3 or 4 facilities; however, no *Aeromonas* was detected in this study, and the U.K. physicians did not use leeches on their amputated patients.[34]

Assessing the combat-related injury extremity infection prevention guidelines pertaining to bacterial infections, key areas of emphasis are early surgery, appropriate antimicrobial therapy without enhanced gram-negative rod coverage or anaerobic activity, and adjunctive topical therapy.[8,31] This strategy appeared to be effective in all cases against initially colonizing bacteria in the wound except in one patient

(subject 9) who underwent surgery within 105 minutes of injury with amoxicillin/clavulanate given within 58 minutes from time of injury. Although the *E. cloacae* found in this patient was resistant to amoxicillin/clavulanate and cefazolin, the wound also remained grossly contaminated on postinjury day 2 at BAF with substantial wound necrosis possibly mitigating the utility of antibiotics. Despite all patients being managed within the 6-hour period recommended in the guidelines, it is unclear with this small sample size and lack of a comparison group whether changes in time to initial debridement would have impacted infectious outcomes. Given the changing pathogens over time among these wounds, the initial antimicrobial therapy may be less relevant than a focus on early surgical control of wound necrosis and contamination.

Molds were found at Role 4 and 5 facilities but not Role 3. It is unclear if this is a sampling bias versus the wound characteristics of necrotic tissue and contamination that could encourage mold growth and sporulation.[24,35,36] Notably, this study had growth of molds in a wound swabs not associated with an amputation transported to the United States (data not shown as they were from other extremity injuries not part of amputation injuries). It is also unclear what role polymicrobial wound contamination with bacteria and fungus plays, as was frequently observed in this series of patients. There is also an unmeasurable potential bias of pathogen detection by routine fungal screening at the Role 4 facility, where a "wound blast protocol" was ongoing during this study. This clinical practice protocol was intended to improve the rapid recovery of fungal pathogens in patients with epidemiologic risk factors for invasive fungal infection, allowing for earlier detection and initiation of therapy.[36] Dedicated in-theater use of Dakin's solution to prevent mold infection was of unclear benefit.[37] Overall, continued investigations into the source of molds and optimal antifungal therapy are needed to improve clinical care.

A limitation of this study is that the patients represented a severely injured population, but this also allows for a greater detection of pathogens given their increased risk for infection. Therefore, one must be cautious in applying these findings to those with less severe injuries. In addition, the sample size is small, but the risk factors for infection and outcomes are similar to previous published studies. There is also the challenge of adequately detecting a pathogen in a combat zone and varying lab capabilities across the military health system potentially favoring fungal detection at Role 4/5 versus Role 3. However, we previously found that bacteria can remain viable on swabs up to 4 weeks at room temperature.[38] Finally, management strategies change over time as do exposures in the combat zone, so applying the lessons from this study to other regions of the world is probably limited, as seen with *Acinetobacter* in Iraq versus Afghanistan.

Overall, mangled lower extremity injuries were found to have a high early contamination rate with environmental organisms that were not associated with sustained infections during the course of the patient's care. In addition, molds can be found in combat-related wounds by swab culture but were not observed in cultures taken at the Role 3 facility. Best practices for combat casualty care should include continual reassessment of changing treatment strategies and epidemiological shifts in a combat zone to ensure optimal care is being provided.

ACKNOWLEDGMENTS

We are indebted to the USAISR Joint Combat Casualty Research Team, to the USAISR Laboratory Support Branch, to the data managers of the Joint Trauma System, and to the USAISR Regulatory Compliance Division. The authors acknowledge the Department of Defense Trauma Registry and Patient Administration Systems and Biostatistics Activity for providing data for this study. Internal funds of the USAISR Clinical Trials in Burns and Trauma Task Area were used to purchase wound swabs, and the Task Area provided internal funds to the USAISR Laboratory Support Branch for microbiological assessment of wound swab specimens and to the USAISR Joint Combat Casualty Research Team to store and ship wound swab specimens.

REFERENCES

1. Eastridge BJ, Mabry RL, Seguin P, et al: Death on the battlefield (2001-2011): implications for the future of combat casualty care. J Trauma Acute Care Surg 2012; 73(6 Suppl 5): S431–7.
2. Krueger CA, Wenke JC, Ficke JR: Ten years at war: comprehensive analysis of amputation trends. J Trauma Acute Care Surg 2012; 73(6 Suppl 5): S438–44.
3. Tribble DR, Conger NG, Fraser S, et al: Infection-associated clinical outcomes in hospitalized medical evacuees after traumatic injury: Trauma Infectious Disease Outcome Study. J Trauma 2011; 71(Suppl 1): S33–42.
4. Owens BD, Kragh JF Jr., Macaitis J, Svoboda SJ, Wenke JC: Characterization of extremity wounds in Operation Iraqi Freedom and Operation Enduring Freedom. J Orthop Trauma 2007; 21(4): 254–7.
5. Napierala MA, Rivera JC, Burns TC, et al: Infection reduces return to duty rates for Soldiers with Type III open tibia fractures. J Trauma 2014; 77(3 Suppl 2): S194–7.
6. Tennent DJ, Wenke JC, Rivera JC, Krueger CA: Characterisation and outcomes of upper extremity amputations. Injury 2014; 45: 965–9.
7. Armed Forces Health Surveillance Center (AFHSC): Amputations. MSMR 2014; 21(3): 23.
8. Murray CK, Obremskey WT, Hsu JR, et al: Prevention of infections associated with combat-related extremity injuries. J Trauma 2011; 71(2 Suppl 2): S235–57.
9. Gustilo RB, Mendoza RM, Williams DN: Problems in the management of type III (severe) open fractures: a new classification of type III open fractures. J Trauma 1984; 24(8): 742–6.
10. Patzakis MJ, Wilkins J: Factors influencing infection rate in open fracture wounds. Clin Orthop Relat Res 1989; 150(5): 36–40.
11. Gustilo RB, Gruninger RP, Davis T: Classification of type III (severe) open fractures relative to treatment and results. Orthopedics 1987; 10(12): 1781–8.
12. Parrett BM, Matros E, Pribaz JJ, Orgill DP: Lower extremity trauma: trends in the management of soft-tissue reconstruction of open tibia-fibula fractures. Plast Reconstr Surg 2006; 117(4): 1315–22.
13. Rajasekaran S, Naresh Babu J, Dheenadhayalan J, et al: A score for predicting salvage and outcome in Gustilo type-IIIA and type-IIIB open tibial fractures. J Bone Joint Surg Br 2006; 88(10): 1351–60.
14. Naique SB, Pearse M, Nanchahal J: Management of severe open tibial fractures: the need for combined orthopaedic and plastic surgical treatment in specialist centres. J Bone Joint Surg Br 2006; 88(3): 351–7.
15. Giannoudis PV, Papakostidis C, Roberts C: A review of the management of open fractures of the tibia and femur. J Bone Joint Surg Br 2006; 88(3): 281–9.

16. Gustilo RB, Anderson JT: Prevention of infection in the treatment of one thousand and twenty-five open fractures of long bones: retrospective and prospective analyses. J Bone Joint Surg Am 1976; 58(4): 453–8.
17. Sen C, Eralp L, Gunes T, Erdem M, Ozden VE, Kocaoglu M: An alternative method for the treatment of nonunion of the tibia with bone loss. J Bone Joint Surg Br 2006; 88(6): 783–9.
18. Lee J: Efficacy of cultures in the management of open fractures. Clin Orthop Relat Res 1997; 339: 71–5.
19. Carsenti-Etesse H, Doyon F, Desplaces N, et al: Epidemiology of bacterial infection during management of open leg fractures. Eur J Clin Microbiol Infect Dis 1999; 18(5): 315–23.
20. Burns TC, Stinner DJ, Mack AW, et al: Microbiology and injury characteristics in severe open tibia fractures from combat. J Trauma Acute Care Surg 2012; 72(4): 1062–7.
21. Yun HC, Branstetter JG, Murray CK: Osteomyelitis in military personnel wounded in Iraq and Afghanistan. J Trauma 2008; 64(2 Suppl): S163–8.
22. Hospenthal DR, Green AD, Crouch HK, et al: Infection prevention and control in deployed military medical treatment facilities. J Trauma 2011; 71(2 Suppl 2): S290–8.
23. Weintrob AC, Murray CK, Lloyd B, et al: Gram-negative multidrug-resistant organism active surveillance for colonization amongst injured service members: a three-year evaluation. MSMR 2013; 20(8): 17–22.
24. Weintrob AC, Weisbrod AB, Dunne JR, et al: Combat trauma-associated invasive fungal wound infections: epidemiology and clinical classification. Epidemiol Infect 2014; 18: 1–11.
25. Vento TJ, Cole DW, Mende K, et al: Multidrug-resistant gram-negative bacteria colonization of healthy US military personnel in the US and Afghanistan. BMC Infect Dis 2013; 13: 68.
26. Mende K, Beckius ML, Zera WC, et al: Phenotypic and genotypic changes over time and across facilities of serial colonizing and infecting *Escherichia coli* isolates recovered from injured service members. J Clin Microbiol 2014 (in press).
27. Sutter DE, Bradshaw LU, Simkins LH, et al: High incidence of multidrug-resistant gram negative bacteria recovered from Afghan patients at a deployed US military hospital. Infect Control Hosp Epi 2011; 32(9): 854–60.
28. Tong MJ: Septic complications of war wounds. JAMA 1972; 219(8): 1044–7.
29. Murray CK, Roop SA, Hospenthal DR, et al: Bacteriology of war wounds at the time of injury. Mil Med 2006; 171(9): 826–9.
30. Brown KV, Murray CK, Clasper J: Infectious complications of combat-related extremity injuries in the British Military. J Trauma 2010; 69(Suppl 1): S109–15.
31. Lloyd BA, Weintrob AC, Hinkle MK, et al: Adherence to published antimicrobial prophylaxis guidelines for wounded service members in the ongoing conflicts in Southwest Asia. Mil Med 2014; 179(3): 234–8.
32. Janvier F, Helacour H, Tesse S, et al: Faecal carriage of extended-spectrum β-lactamase-producing enterobacteria among soldiers at admission in a French military hospital after aeromedical evacuation from overseas. Eur J Clin Microbiol Infect Dis 2014. Available at http://link.springer.com.lrc1.usuhs.edu/article/10.1007%2Fs10096-014-2141-8; accessed June 11, 2014.
33. Penn-Barwell J, Fries C, Sargeart I, Bennett P, Porter K: Aggressive soft tissue infections and amputation in military trauma patients. J Roy Nav Med Serv 2012; 98(2): 14–8.
34. Schnabi SM, Kunz C, Unglaub F, Polykandriotis E, Horch RE, Draqu A: Acute postoperative infection with *Aeromonas hydrophila* after using medical leeches for treatment of venous congestion. Arch Orthop Trauma Surg 2010; 130(10): 1328–8.
35. Rodriguez CJ, Weintrob AC, Shah S, et al: Risk factors associated with invasive fungal infections in combat trauma. Surg Infection 2014. Available at http://online.liebertpub.com.lrc1.usuhs.edu/doi/pdf/10.1089/sur.2013.123; accessed June 11, 2014.
36. Lloyd B, Weintrob AC, Rodriguez C, Dunne JR, Weisbrod AB, Hinkle M, et al: Effect of early screening for invasive fungal infections in U.S. Service Members with explosive blast injuries. Surg Infection 2014. Available at http://online.liebertpub.com.lrc1.usuhs.edu/doi/pdf/10.1089/sur.2013.123; accessed June 5, 2014.
37. Barsoumian A, Sanchez CJ, Mende K, et al: In vitro toxicity and activity of dakin's solution, mafenide acetate, and amphotericin B on filamentous fungi and human cells. J Orthop Trauma 2013; 27(8): 428–36.
38. Mende K, Beckius ML, Hospenthal DR: Recovery of multidrug-resistant bacteria from swabs stored for durations of 1 and 4 weeks under conditions mimicking long-distance-shipping conditions. J Clin Microbiol 2014; 52(5): 1798–9.